Development 122, 2631-2638 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV0080

SHORT INTEGUMENT (*SIN1*), a gene required for ovule development in *Arabidopsis*, also controls flowering time

Animesh Ray*, Jean D. Lang, Teresa Golden and Sumita Ray

Department of Biology, University of Rochester, Rochester, NY 14627, USA

*Author for correspondence (e-mail: ray@ar.biology.rochester.edu)

SUMMARY

The *short integument* (*sin1*) mutation causes a female-specific infertility, and a defect in the control of time to flowering in *Arabidopsis*. Female sterility of Sin$^-$ plants is due to abnormal ovule integument development and aberrant differentiation of the megagametophyte in a subset of ovules. An additional defect of *sin1* mutants is the production of an increased number of vegetative leaf and inflorescence primordia leading to delayed flowering. The delayed flowering phenotype of *sin1-1* is not due to a defect in the perception of day length periodicity or in gibberellic acid metabolism. Phenotypes of double mutant combinations of *sin1* with *terminal flower* (*tfl1*) indicate that SIN1 activity is required for precocious floral induction typical in a *tfl1* mutant. Unexpectedly, *sin1-1 tfl1-1* plants do not make pollen, thus revealing a novel role for *TFL1* in the anther. Early flowers of *sin1-1 ap1-1* double mutants are transformed to long inflorescence-like shoots. A genetic model for the role of *SIN1* in flowering time control is proposed.

Key words: *Arabidopsis*, meristem, flowering time, ovule, *short integument*

INTRODUCTION

The form of higher plants is governed by temporal expression of a morphogenetic program that interacts with the environment. In the apical region of the shoot, a set of undifferentiated and rapidly dividing cells, meristem, constitutes a developmental machine that by division and differentiation generates a series of anlagen destined to make specialized organs such as leaves, inflorescence and, finally, flowers. These organs appear in a precise temporal and spatial order in the flanks of dividing meristem cells (Poethig, 1990). The genetic regulation of meristem phase change leading ultimately to flowering is a central problem in plant biology. In *Arabidopsis thaliana*, meristem development progresses through at least three distinct phases: from vegetative (V) through inflorescence (I) to the floral (F) mode (V —› I —› F switch). In *Arabidopsis* this switch is under multiple genetic controls and responds to day length and temperature (Coupland, 1995; Lee et al., 1994; Martinez-Zapater et al., 1994; Sung et al., 1992; Weigel, 1995; Wilson et al., 1992; Yang et al., 1995; Zagotta et al., 1992). A number of genes that participate in the above processes have been identified by their effects on flowering time (Coupland, 1995; Koornneef et al., 1991). Two genes, *EMBRYONIC FLOWER1* and *2* (*EMF1* and *EMF2*) have been proposed to be central negative regulators of flowering, which integrate the effects of environmental factors and thereby control the expression of floral meristem identity genes (Coupland, 1995; Yang et al., 1995).

Three genes, *LEAFY* (*LFY*), *APETALA1* (*AP1*), and *CAULIFLOWER* (*CAL*), are important in the I —› F switch (Bowman, 1992; Gustafson-Brown, 1994; Huala and Sussex, 1992; Irish and Sussex, 1990; Kempin et al., 1995; Mandel and Yanofsky, 1995; Weigel et al., 1992; Weigel and Nilsson, 1995). *AP1* and *LFY* genes are required to induce the transcription of floral organ identity genes *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*) and *AGAMOUS* (*AG*) in the developing floral anlagen (Huala and Sussex, 1992; Ma, 1994; Mandel et al., 1992; Weigel and Meyerowitz, 1993, 1994; Weigel et al., 1992). These genes and their homologues in *Antirrhinum* appear to code for transcription factors (Kempin et al., 1995; Mandel et al., 1992; Weigel et al., 1992; Weigel and Meyerowitz, 1994). In accordance with their inferred role in the floral meristem, *LFY* and *AP1* messages accumulate in the floral but not in the inflorescence meristem (Bowman, 1992; Huala and Sussex, 1992; Weigel et al., 1992). *TFL1* (*TERMINAL FLOWER*) is a proposed negative regulatory gene of *LFY* and *AP1* in the inflorescence meristem (Gustafson-Brown, 1994; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). This is because *tfl1* mutations cause lateral and apical inflorescence meristems to be converted to floral meristem (Alvarez et al., 1992; Shannon and Meeks-Wagner, 1991). Genetic and molecular studies indicate that *TFL1* prevents *LEAFY* and *AP1* activity in the apical and lateral inflorescence meristems (Gustafson-Brown et al., 1994; Shannon and Meeks-Wagner, 1993). Therefore, the current model of the I —› F switch postulates a down regulation of *TFL1* activity that leads to increased expression of *LFY*, *AP1* and *CAL* genes in the flanking meristem.

The evolution of flowering plants may have entailed a modification of primitive leaf or leaf-like structures that contained naked ovules on their surfaces, to specify floral organs that ultimately evolved to surround the ovules (Herr, 1995; Stebbins,

1974). This view of angiosperm evolution predicts that the genetic regulatory network that controls ovule development should be interlaced with that which triggers flowering. Several genes important for ovule development have been identified in *Arabidopsis* (Reiser and Fischer, 1993). *BELL1*, a so-called cadastral gene that encodes a homeodomain protein (Reiser et al., 1995), controls the expression of the floral organ identity gene *AG* within the ovule and thereby controls morphogenesis of ovule integuments (Modrusan et al., 1994; Ray et al., 1994). *SUPERMAN*, another cadastral gene that restricts the spatial expression pattern of the floral organ identity gene *AP3* (Sakai et al., 1995), is important in ovule integument development (Gaiser et al., 1995). The organ identity gene *AP2* is also known to control ovule morphogenesis (Modrusan et al., 1994). By contrast, no known meristem identity or flowering control gene has a demonstrated role in ovule development.

The gene *SHORT INTEGUMENT* (*SIN1*) is required for normal ovule development (Lang et al., 1994; Reiser and Fischer, 1993; Robinson-Beers et al., 1992). We have determined that the *sin1* mutation additionally causes a defect in the V —› I —› F switch. *SIN1* is needed for the expression of the early flowering phenotype imparted by a *tfl1* mutation and *tfl1 sin1* double mutants do not produce pollen. Furthermore, we demonstrate that the *sin1-1* allele enhances the effect of an *ap1* mutation. Thus, *SIN1* represents a genetic connection between ovule development and control of flowering.

MATERIALS AND METHODS

Strains

Strain WC1 was *gl1* Columbia (Co). *sin1 ER* strain was Sin1-C described before (Lang et al., 1994). The source of *tfl1-1* was CS6167 (Co) (Shannon and Meeks-Wagner, 1993), *tfl1-2* was CS3091 in the Landsberg *erecta* background (La-*er*) (Alvarez et al., 1992), *ap1-1* was NW4 (La-*er*) (Lang et al., 1994). The strain containing the *sin1-2* (La-*er*) mutation was generously provided by Dr Charles S. Gasser (University of California, Davis).

Plant growth and physiological conditions

Conditions for germination and growth of *Arabidopsis* have been described before (Lang et al., 1994). Long day photoperiod (LD) was 18 hours light followed by 6 hours of dark. Short day (SD) was 14 hours light and 10 hours dark. To determine the effect of gibberellic acid (GA), a 50 μM solution of GA_3 (Sigma) in 10% acetone was sprayed, with a nebulizer, as a fine mist on the plants on every third day. Control plants were sprayed with 10% acetone. The spraying was done in an open area during the light period. The applied solution was allowed to evaporate for 1 hour at the room temperature before the plants were replaced in the growth chamber.

Genetic techniques

All double mutant combinations except *sin1-1/sin1-2* were constructed by applying pollen from Sin1-C plants to the stigma of the recipient mutants. Wherever possible morphological markers, such as *glabra1* (*gl1*: absence of trichomes on leaf), *erecta* (*er*: compact rosette leaves, short pedicel, blunt fruits) and *chlorina* (*ch1*, light green color of leaves and stems), were monitored for segregation in subsequent progeny to verify the crosses. Double or triple (involving *gl1*) mutants were identified in F_2 by their respective phenotypes. Unless stated explicitly, only those progenies that were phenotypically Er^+ were analyzed. Allelism test between *sin1-1* and *sin1-2* was done by crossing pollen from Sin1-C plants into phenotypically Sin^+ plants in a La-*er* population segregating the *sin1-2* allele. F_1 seeds derived from individual crosses were separately germinated and tested for the appearance of Sin^- progeny with an Er^+ phenotype. One out of the five independent crosses tested produced 6 Sin^-:7 Sin^+ F_1 progeny (expect 1:1 for non-complementation), all of which were Er^+, establishing allelism. The Sin^- plants produced in this batch of F_1 were *sin1-1/sin1-2*.

Microscopic techniques

Techniques for tissue preparation and scanning electron microscopy were as described before (Lang et al., 1994).

RESULTS

sin1 mutation causes a defect in programmed meristem phase change

When grown under the long day condition, wild-type *Arabidopsis* initially produces a succession of five to eight (seven, on average) vegetative leaves in a helical arrangement from meristematic cells set aside in the flanks of the primary apical meristem (Fig. 1; Table 1). Next, two to three inflorescence meristem clusters, each subtended by a cauline leaf (or bract), initiate from the flanks of the growing apical meristem. These inflorescence primordia are termed coflorescences as they are borne on the flanks of the main inflorescence axis (Schultz and Haughn, 1993). Cells of the vegetative internode, the column of cells between two successive leaves, do not expand appreciably while internodal cells of the inflorescence (those between two successive coflorescence axes, and those between two flowers) usually do expand. Finally, a series of flowers without subtending bracts initiates from the flanking lateral meristem. The first leaf primordium appears on day 5 and the inflorescence axis starts to elongate on day 18. By day 18, at least five floral buds have formed. Thus, organ primordia appear at an average rate of approximately one per day until fertilization begins. Secondary inflorescence branches appear at a later stage from axils of rosette leaves. Since morphogenesis of these secondary inflorescences can be variable, we will not consider these any further.

Plants homozygous for the *sin1-1* mutation (Fig. 1C) produce approximately 16 rosette leaves, 13 inflorescence primordia with subtending bracts, and five to six floral buds before starting to expand shoot internodes on the 40th day (Table 1). Thus, the meristem initials in the *sin1* mutants appear at the same rate of approximately one per day as for a Sin^+ plant, but the duration spent in each phase of development is at least doubled compared to the wild type. The resulting plants are taller and are more luxuriant. The total number of flowers borne on a wild-type or on a *sin1-1* plant is indefinite: the wild type usually bears fewer flowers than *sin1-1* on the

Table 1. Effect of *sin1* mutation on meristem phase-change

Strain	Treatment	Days to 1 cm bolt (± s.e.)	Number of rosette leaves (± s.e.)	*n*	Number of coflorescence (± s.e.)	*n*
WC1	LD	20.4 (±0.2)	7.6 (±0.1)	33	3.4 (±0.1)	44
sin1	LD	39.6 (±0.5)	19.2 (±1.2)	46	15.3 (±0.7)	46
WC1	LD GA_3	18.2 (±1.7)	7.0 (±0.2)	49	2.9 (±0.2)	29
sin1	LD GA_3	30.4 (±1.9)	12.4 (±2.0)	49	19.4 (±1.3)	30

n = number of plants tested.

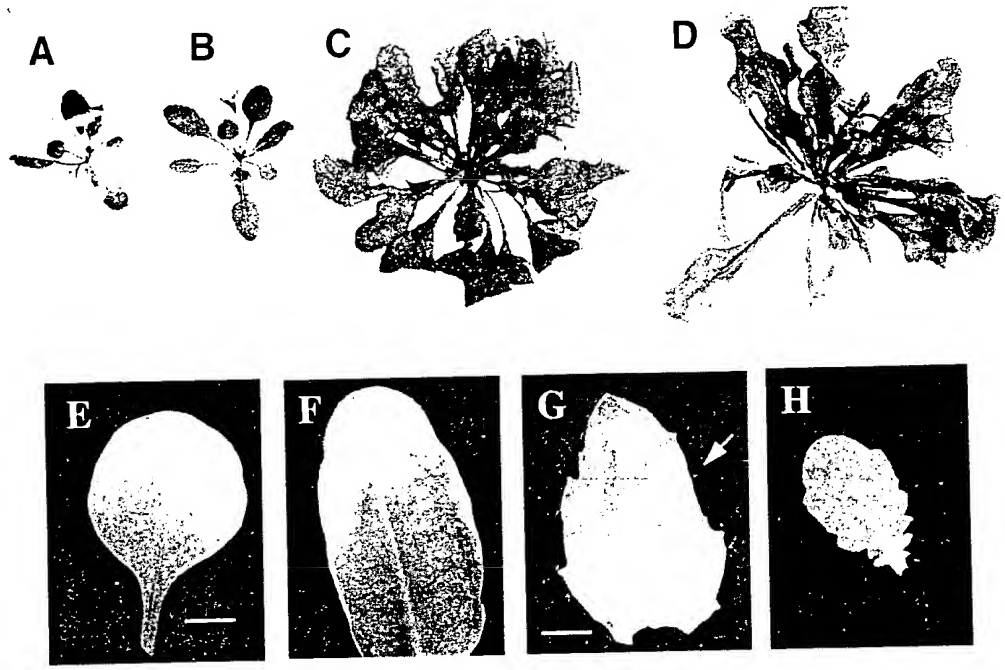

Fig. 1. Effects of mutations on vegetative development. A-D are photographs of plants grown in long days until the inflorescence shoots reached approximately 1-2 cm. (A) *gl1 tfl1-1*; (B) strain WC1, mutant for *gl1* but otherwise wild type; (C) *gl1 sin1-1*: (D) *gl1 sin1-1 tfl1-1*. E-H are photographs of leaves of a WC1 plant (grown in SD to make more than 13 rosette leaves). E,F and H show the second, seventh and the 13th rosette leaf, respectively, with no trichomes on leaf margin. G shows a cauline leaf with trichomes (arrow) which subtends a coflorescence shoot. Scale bars: (E,F,H) 0.4 cm; (G) 0.2 cm.

primary inflorescence (the main shoot) because successful fertilization in the wild type causes a cessation of flowering, while the mutant, being female sterile, flowers indefinitely. The apical meristem in both the wild type and the mutant remains indeterminate (Fig. 2A,B).

A second allele of *sin1* (designated *sin1-2*) has the same associated phenotypes of female sterility and meristem transition defects. Allelism test (see Methods) confirmed that *sin1-2* does not complement *sin1-1* in any of the phenotypes. First, *sin1-2* mutant plants are late flowering and produce many more rosette leaves and coflorescences compared to the wild type. Second, *sin1-2* mutants are female sterile because of ovule defects. The ovule defect in *sin1-2* was less severe in morphological terms than those associated with *sin1-1* allele. The ovule defect of *sin1-1/sin1-2* heterozygotes was of intermediate severity. The phenotypic series for ovule defect is *sin1-1* > *sin1-1/sin1-2* > *sin1-2*. Therefore, both *sin* alleles are loss-of-function alleles. There was, however, no significant difference in flowering time between homozygous *sin1-1* and *sin1-2* mutants: 36±1 days (n=16) for 1 cm bolt with *sin1-2* compared to 39±0.5 days (n=46) for *sin1-1*. *sin1-1/sin1-2* heterozygous plants were approximately equally affected for flowering time (33±1 days, n=7) and the number of organs produced (number of rosette leaves plus coflorescence on the primary axis = 30±1, n=7). Thus the meristem transition phenotype does not show the same phenotypic series as shown by the ovule phenotype. One interpretation of these results is that meristem transition is more sensitive to the level of SIN1 product than is ovule morphogenesis.

Environmental effects on *sin1-1* phenotype

Several classes of late flowering mutants of *Arabidopsis* show an altered response to day length and/or to the plant hormone, gibberellic acid (GA) (Coupland 1995; Koornneeff et al.,

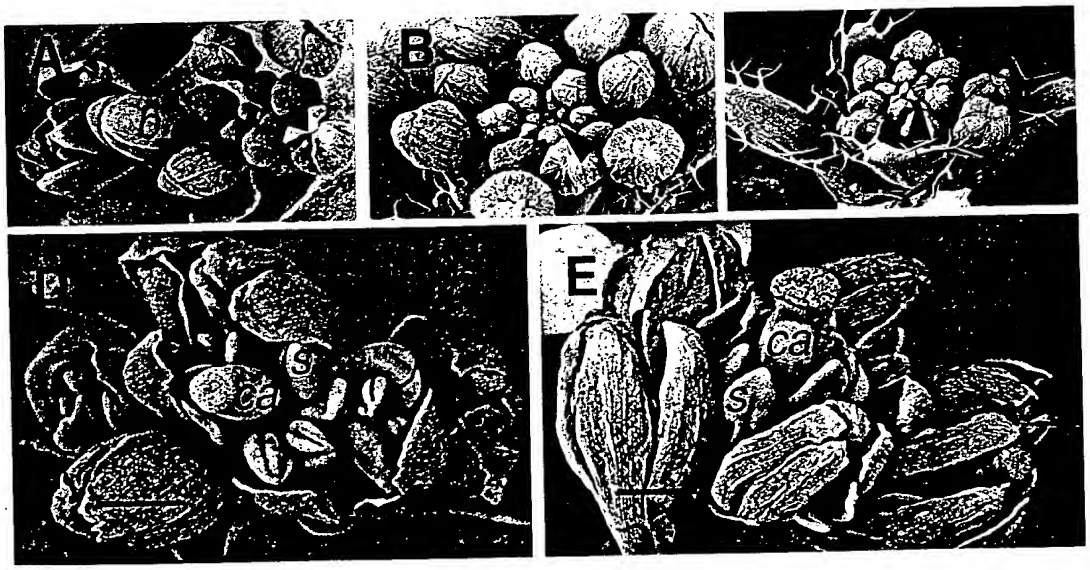

Fig. 2. Scanning electron micrographs of inflorescence apices. Mature inflorescence tips are shown in (A) WC1; (B) *sin1-1*: (D) *tfl1-1*; and (E) *sin1-1 tfl1-1*. C shows an immature apex of a *tfl1-1* mutant. Arrowheads in A and B point to the undifferentiated apical meristem dome. In C, the arrowhead points to the developing carpel primordium of the terminal flower. Abbreviations: *b*, a flower bud; *ca*, carpel. Scale bars are 0.25 mm in all panels.

1991). In fact, phenotypic analysis of the *sin1-1* mutant had earlier led us to propose that it may be defective in some aspect of GA metabolism. The following observations make this possibility unlikely. Application of GA_3 did not reverse the late flowering phenotype but accelerated flowering of *sin1-1* growing in long days (LD) to approximately the same extent as it did to the wild type (Table 1). Growth of *sin1-1* in short days (SD) prolonged the flowering time to 280±15 days (number of plants, n=8) compared to 87±5 days (n=29) for the wild type in SD. The ratio of flowering times of the mutant to the wild type was not significantly different in short and long days. Repeated applications of GA_3 accelerated the flowering time of Sin^- plants in SD as it did to Sin^+ plants (data not shown). Both Sin^+ and Sin^- plants responded to GA_3 by growing longer and more slender leaves and shoots. Both Sin^+ and Sin^- plants treated with GA_3 were partially male sterile. In summary, floral induction in *sin1-1* plants was responsive to both day length periodicity and gibberellic acid treatment. Vernalization, cold treatment of hydrated seeds before germination, had no effect on flowering time. These results suggest that the *sin1-1* mutation does not directly cause a defect in photoperiod response, and in metabolism of or signal transduction by GA.

Origin of extra organs in *sin1-1*

The delayed meristem transition phenotype of *sin* mutants may be due to a defect in the internal developmental program. The extra rosette leaves in a *sin1* mutant may represent organs normally found associated with the inflorescence but which are now converted to vegetative leaves. If true, it may be possible to obtain a morphological marker that is normally specific for an inflorescence organ but may appear ectopically in the supernumerary rosette leaves of *sin1-1* mutant plants. The number and density of trichomes on the abaxial surface of rosette leaves change with developmental time: late-appearing leaves have more abaxial trichomes than early leaves (Schultz and Haughn, 1993). This criterion has previously been used to mark developmental time (Schultz and Haughn, 1993; Weigel and Nilsson, 1995). By contrast, we wished to use an organ-specific marker that never appears on rosette leaves, but does so on cauline leaves (bracts) that subtend a coflorescence branch off the main inflorescence shoot. We noted that trichomes, absent on the edges of rosette leaves in a *gl1* mutant growing in LD, are always present on bract margins (Figs 1E-H, 3; Table 2). Leaf margin trichomes do not appear until the thirteenth leaf of a *gl1* plant growing in SD, and a few appear on subsequent leaves. By contrast, trichomes first appear on the edge of the eighth leaf of a *sin1-1 gl1* double mutant growing in LD and appear at increasing density on the edges of subsequent leaves (Fig. 3). Thus, by the single criterion of trichomes on rosette leaf edge in a *gl1* mutant background, the extended vegetative phase in the *sin1-1* mutant is correlated with at least a partial operation of an inflorescence-like program in which bract-specific trichomes appear on rosette leaf margins.

Leaf shape was used in other related studies to monitor developmental transition in *Arabidopsis* (Schultz and Haughn, 1993). We could not detect a sharp transition in our metric for leaf shape (Fig. 3) between the seventh and the eighth leaf; hence a change in leaf shape was not a reliable marker of developmental transition in these strains.

Table 2. Interaction of *sin1* with *tfl1*

Strain	Days to 1 cm bolt (± s.e.)	Number of rosette leaves: Without trichome (± s.e.)	Number of rosette leaves: With trichome (± s.e.)	Number of rosette leaves: Total	Number of coflorescence (± s.e.)	n
WC1	23.6 (±0.4)	6.5 (±0.1)	0	6.5	2.2 (±0.1)	48
sin1 gl1	41.7 (±1.7)	8.5 (±1.0)	16.8 (±1.6)	25.3	8.3 (±0.7)	13
tfl1-1 gl1	21.6 (±0.1)	5.9 (±0.2)	0	5.9	1.0 (±0.1)	20
tfl1-2	18.5 (±0.3)	0	4.3 (±0.1)	4.3	1.0 (±0.1)	16
tfl1-1 sin1 gl1	39.3 (±0.5)	5.4 (±0.2)	13.3 (±1.6)	18.7	7.2 (±0.4)	21
tfl1-2 sin1 gl1	32.3 (±0.7)	10.7 (±0.6)	10.2 (±1.2)	20.9	4.2 (±0.4)	6

The *sin1* allele tested was *sin1-1*. n is the number of plants tested. All plants were grown in LD.

Tfl^- phenotype is modified by *sin1* mutation

The mutation *tfl1* causes early flowering, a reduction in the number of lateral inflorescence primordia (coflorescence), and produces a terminal differentiation of the apical meristem to a floral meristem (Alvarez et al., 1992; Shannon and Meeks-Wagner, 1991, 1993). In the sense that the *tfl1* mutation pre-

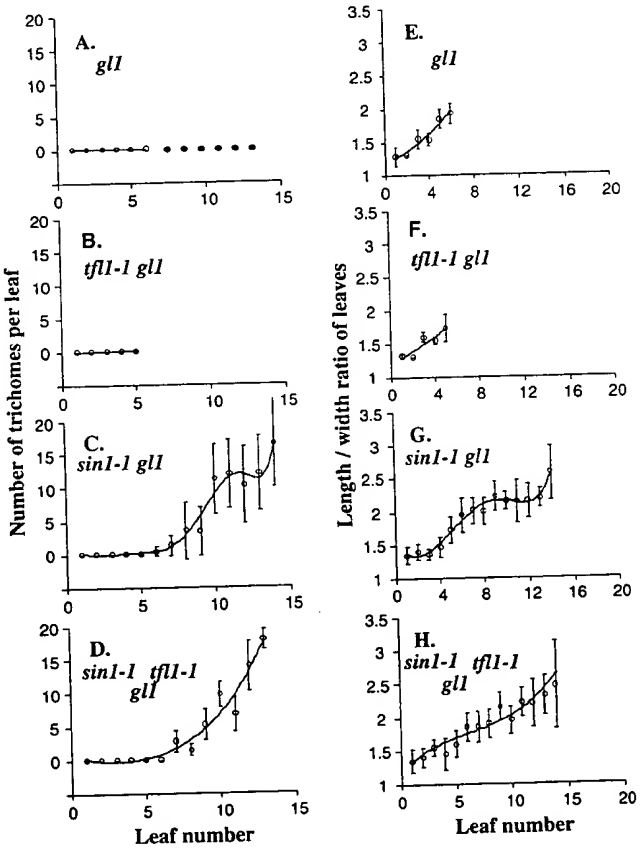

Fig. 3. Variation in the number of leaf-edge trichomes, and leaf shape throughout development. The developmental time is represented by rosette leaf number in the order of appearance: leaf 1 is the earliest leaf. All data are from plants grown simultaneously in LD, except those denoted by filled circles in A which were grown in SD. Error bars represent standard errors of mean. Curves were drawn by fitting a polynomial through the means.

maturely commits the vegetative meristem to reproductive development, *sin1* causes an opposite effect. *sin1* is pleiotropic on meristem transition during both vegetative and inflorescence phases and on ovule morphology. It has been argued elsewhere that ovules are independent floral organs (Colombo et al., 1995; Herr, 1995; Meyerowitz, 1994; Ray et al., 1994; Taylor and Taylor, 1993). The *TFL1* gene negatively regulates the activities of other genes, such as *APETALA1* and *LFY*, that are important for both floral meristem identity and floral organ identity (Gustafson-Brown et al., 1994; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993; Weigel et al., 1992). Therefore it is interesting to determine if *TFL1* also controls *SIN1*.

In the LD growth regime, the Sin$^+$ strain WC1 produced on average seven rosette leaves and two coflorescence branches (identified by subtending bracts) that in turn bore secondary and tertiary coflorescence primordia (Table 2). Homozygous *sin1-1* mutant plants under an identical condition produced on average 25 rosette leaves and eight coflorescence branches (Table 2). The apical inflorescence meristem of WC1 and *sin1-1* plants remained indeterminate (Fig. 2A,B): compared to WC1, many more organ primordia were arranged in a spiral motif around the central dome of the apical meristem in the *sin1* mutant. We tested two mutant alleles of *tfl1*. Each *tfl1* mutant plant in LD generated on average four to six rosette leaves and one bract that subtended a flower, not a coflorescence (Fig. 1; Table 2). The inflorescence apices differentiated to one or more terminal flowers (Fig. 2C,D).

In LD condition, double mutants of *tfl1-1 sin1-1* and *tfl1-2 sin1-1* generated on average 19 to 21 rosette leaves, and four to seven coflorescence branches, many of which bore several secondary coflorescence primordia (Table 2; Fig. 1D). However, the apex of each inflorescence (and coflorescence) of the double mutant plants differentiated into terminal flowers (Fig. 2E).

Consistent with our idea that supernumerary rosette leaves in strains involving the *sin1* mutation have partial inflorescence characteristics, the extra leaves of *tfl1 sin1 gl1* triple mutant plants displayed many trichomes on their margins (Table 2; Fig. 3D).

Besides reversing the early flowering phenotype of *tfl1* mutation, the *sin1 tfl1* double mutations produced female sterility due to ovule defects typical of a *sin1-1* single mutant. Ovule morphology of *tfl1 sin1-1* double mutants was identical to those of *sin1-1* single mutants (data not shown). Unexpectedly, the double mutants were completely male sterile: the androecium development was morphologically normal (Fig. 2E) but the anthers remained white and failed to develop pollen grains. This synergistic interaction between *sin1* and *tfl1* revealed a cryptic role for *TFL1* in pollen development. A cryptic role for *SIN1* in pollen development was reported earlier (Lang et al., 1994; Robinson-Beers et al., 1992).

sin1 interacts with *ap1* mutation

Since *TFL1* is postulated to regulate meristem transition at least partially through its action on *AP1* (Gustafson-Brown et al., 1994; Shannon and Meeks-Wagner, 1993), we investigated whether *SIN1* also does so. If *SIN1* is a positive regulator of *AP1*, the effect of an *ap1* mutation should be enhanced in a *sin1 ap1* double mutant.

The strong mutant allele *ap1-1* (in a background wild type for *ER*) produced a normal number of rosette organs and coflorescences but its flowers had partial inflorescence characteristics (Schultz and Haughn, 1993; Table 3; Fig. 4A). The floral stalk, as usual, was not subtended by a bract; in place of a sepal often there was a bract and a flower. Each floral stalk in the *ap1-1* mutant had at most five flowers, four originating from the site of four sepals and one that represented the main floral axis. The number of supernumerary flowers borne on a floral stalk decreased acropetally. These supernumerary flowers were arranged in a radial symmetry, a characteristic of floral organs, as opposed to the helical symmetry of flower series along the main inflorescence axis. On the basis of these characteristics, it is thought that the supernumerary flowers of *ap1* are transformed floral organs (Ma, 1994).

In a *sin1-1 ap1-1* double mutant (in *ER* background), the number of rosette organs and coflorescence primordia is increased over the wild type to the same extent as in a *sin1-1* single mutant (at both 17°C and 23°C; data not shown). This is consistent with previous observations that AP1 has no role in vegetative or inflorescence primordia (Bowman et al., 1993; Schultz and Haughn, 1993). In contrast to the *ap1-1* single mutant, early-arising floral primordia in more basal positions assume strong inflorescence-like characteristics: the floral stalk (identified by the absence of a bract; Fig. 4B) elongates like an inflorescence shoot and often bears a series of bracts, coflorescence primordia and flowers in a helical array (Table 3). These modified floral structures ultimately terminate in a multiple-flower, typical of the *ap1-1* mutant flower, suggest-

Table 3. Interaction between *sin1-1* and *ap1-1* mutations

Temperature	Strain	Plant number	Flowers that are: Inflorescence-like	Flowers that are: Canonical Ap1$^-$	First normal flower
23°C	*ap1-1*	1	1st	2nd to 12th	13th
		2	–	1st to 12th	13th
		3	–	1st to >10th	†
		4	–	1st to >10th	†
		5	–	1st to >10th	†
		6	–	1st to >10th	†
		7	–	1st to >10th	†
		8	–	1st to >10th	†
		9	–	1st to 7th	8th
23°C	*ap1-1 sin1-1*	1	1st to 14th	15th to 21st	22nd
		2	1st to 21st	22nd to 30th	>30th
		3	1st to 4th	5th to 20th	21st
		4	1st*	2nd to 16th	17th
		5	–	1st to 23rd	24th
		6	–	1st to 5th	6th
17°C	*ap1-1*	1	–	1st to 11th	12th
		2	–	1st to 15th	16th
		3	–	1st to 9th	10th
		4	–	1st to 8th	9th
17°C	*ap1-1 sin1-1*	1‡	1st to 29th	30th	31st
		2	1st to 43rd	44th to 62nd	63rd

*The structure that occupied the position of the first flower (the first organ primordium without a subtended bract) was a determinate inflorescence with 29 nodes and two expanded secondary coflorescences with 3 and 16 nodes, respectively.

‡Positions occupied by the first to the eighth flowers were structures with strong inflorescence characteristics with gradual acropetal lessening of severity of the transformation. Positions 13th through 29 were occupied by structures strongly resembling inflorescences.

†Not recorded. All plants were grown in LD.

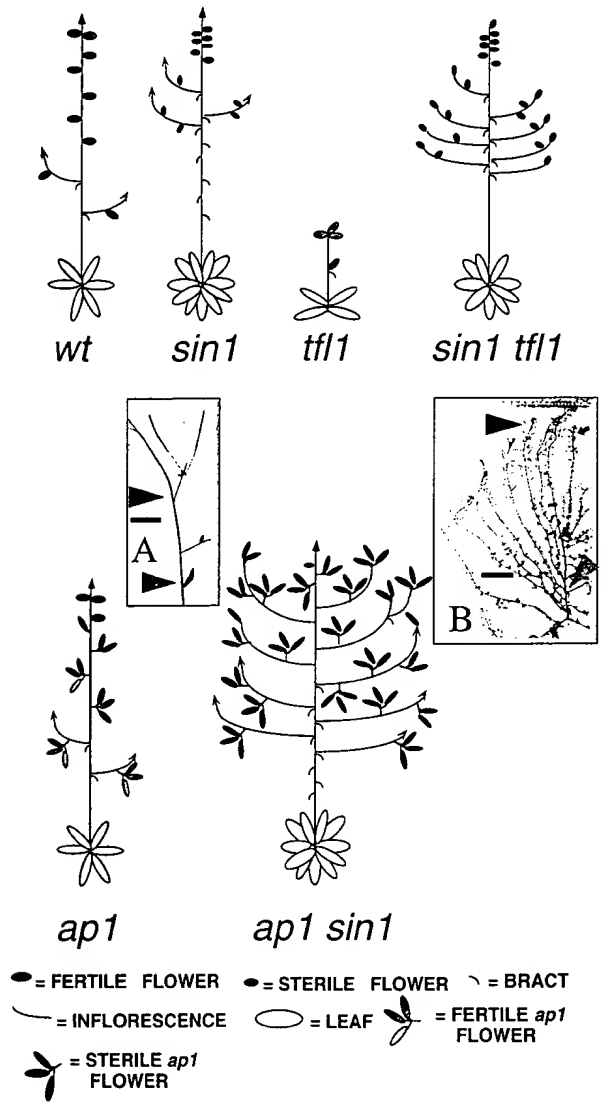

Fig. 4. Summary of inflorescence phenotypes of mutant plants. Arrows on inflorescence apices represent indeterminate apical meristems that continue to grow without final differentiation. *sin1* causes the appearance of more rosette organs and coflorescences than in the wild type. A *tfl1* mutant has fewer while a *sin1 tfl1* double mutant has more rosette leaves and coflorescences than the wild type. In the *ap1* mutant, the number of rosette leaves and coflorescences are equal to that in the wild type, while a *ap1 sin1* double mutant makes more rosette leaves and coflorescences and has inflorescence-like organs in place of Ap1$^-$ flowers. All plants with a *sin1* mutation are female sterile; *sin1 tfl1* double mutants are both female and male sterile. The inset A shows a portion of a typical *ap1-1* plant. The black arrowhead points to the second flower on the primary inflorescence axis. The gray arrowhead points to the last coflorescence branch, as identified by the presence of a subtending bract. The inset B shows a portion of a typical *ap1-1 sin1-1* plant. The arrow points to the apex of the primary inflorescence (indeterminate). The black arrowhead points to the apex of a long inflorescence-like organ that should have been the first flower along the primary inflorescence axis. This modified flower produced over 13 coflorescence primordia, two secondary coflorescence shoots and innumerable Ap1 flowers before terminating in a single flower. Scale bars: 1 cm in A and 4 cm in B.

ing that floral characteristics eventually dominate in these modified organs. The severity of the *sin1-1* effect on *ap1-1* decreases acropetally, such that late-arising flowers are less inflorescence-like, and later flowers resemble the canonical *ap1-1* mutant flowers (Table 3). Since the *ap1-1* mutant is known to be cold sensitive, we grew and observed mutant plants at 17°C. The first flowers of the double mutants were more severely affected at 17°C than those in the *ap1-1* single mutant (Table 3). For example, in one *ap1-1 sin1-1* double mutant plant, an elaborate inflorescence with over a hundred organs developed in place of a flower. Thus, the *sin1-1* mutation enhanced the effect of *ap1-1*.

DISCUSSION

The basis of late flowering in *sin1*

The late flowering phenotype of *sin1* may be interpreted in one of several ways. First, as is the case with several late flowering mutants, the *sin1* mutation may cause a defect in the transduction of environmental signals. For example, the *co*, *gi*, and the *fha* mutations cause a delay in flowering when grown in long days but not in short days (Koornneef et al., 1991; Martinez-Zapater et al., 1994; Putterill et al., 1995). The *sin1* mutation does not belong in this category of mutations because growth in short days causes a further delay when compared to long day growth: while Sin$^+$ plants flowered after approximately 80 days, *sin1* mutants flowered after approximately 270 days. Short day grown *sin1* mutant plants develop hundreds of vegetative leaves, more than 50 coflorescences, and grow to gigantic proportions. Late flowering phenotypes imparted by *fy*, *fpa*, *fve*, *fca*, *ld*, *fe*, and *fri* mutations can be reversed by vernalization (Clarke and Dean, 1994; Koornneef et al., 1991). Vernalization had no effect on the late flowering phenotype of *sin1*, so it cannot be included in this class. Repeated applications of gibberellic acid on vegetative leaves failed to rescue the late flowering phenotype of *sin1* mutants grown in long day conditions. In short days, repeated gibberellic acid application accelerated the time to flowering of both the wild type and *sin* plants. Thus, *sin1* mutation endows the plants with neither a gibberellin requirement nor a gibberellin insensitivity. Superficially, *sin1* resembles a class of poorly understood mutations, including *fwa*, *fd* and *ft*, that are late flowering in both long and short days and are insensitive to vernalization (Koornneef et al., 1991). Note, however, that *fwa* is a dominant mutation while *sin1* is recessive. Furthermore, none of these other three mutations has any effect on fertility. Therefore, the *sin1* mutation defines a novel class by its phenotypic effects: late flowering in both long and short days, cannot be rescued by gibberellic acid or vernalization, and the associated female sterility. The map location of *sin1* (at 1.0 cM from *an* on chromosome 1; Lang et al., 1994) rules out the possibility that it is an allele of any known late flowering mutation that has been mapped so far.

Alternatively, *sin1* may be a mutation in a meristem identity gene in the same sense as is the *leafy* mutation. While the *lfy* mutation affects only the transition from the inflorescence to the floral meristem, *sin1* affects both V $\longrightarrow$ I and I $\longrightarrow$ F switches. Thus, on average in LD, the fates of the eighth to the 16th organ primordia in a *sin1* mutant are changed from that of inflorescences or of flowers to that of leaves, and the fates of

the 17th to the 20th primordia are changed from that of flowers to inflorescences. Consistent with this interpretation, supernumerary rosette leaves of *sin1 gl1* and *sin1 tfl1 gl1* plants contain trichomes on their margins, as do the cauline leaves (bracts) but not the seven vegetative leaves of *SIN1 gl1* plants.

Interaction of *SIN1* with *AP1* and *TFL1*

In a *sin* mutant, the meristem appears to spend a longer time at each developmental phase. It is formally possible that the wild-type SIN product encodes a general positive regulator of meristem phase change. The absence of epistasis of *tfl1* on *sin1* (Fig. 4) allows us to reject the simple possibility that *SIN1* is a negative regulator of *TFL1*. *SIN1* may act through a genetic pathway independent of *TFL1*. In that case, since *tfl* has an effect opposite to that of *sin1* on the delayed flowering phenotype, *tfl1 sin1* double mutants should be intermediate between the two single mutants. Thus, we expect on average nine supernumerary leaves (with trichomes on the leaf margin) and five coflorescence primordia in the double mutant growing in LD. The observed results are not significantly different (Table 2). Furthermore, each apical meristem ultimately developed into a terminal flower. Therefore, I —› F transition at the inflorescence apex does not require *SIN1* activity. While *SIN1* may act independently of *TFL1*, it could still be acting through *AP1* and *LFY*. A further possibility is that *TFL1* indirectly controls *LFY* and *AP1* expressions through its effect on *SIN1*. The predicted outcome is that *sin1* should be epistatic over *tfl1*. This is partially true. In LD, a *sin1 tfl1* double mutant plant produces on average 25-26 non floral organs as opposed to only 5-7 in *tfl1* single mutants and approximately 33 in *sin1* single mutants. The values for *sin1* and *tfl1 sin1* mutants overlap at the 99% confidence level (Table 2), and are not significantly different. Each coflorescence of the double mutant in turn develops several secondary coflorescence primordia and many female sterile flowers (Fig. 4), unlike what is observed for a *tfl1* mutant. In summary, *sin1* is a partial suppressor of *tfl1*, and *TFL1* may regulate meristem fate through *SIN1*.

The phenotype of a *sin1-1 ap1-1* double mutant (Fig. 4) is significantly different from that of an *ap1 cal* double mutant where the inflorescence meristem fails to extend and the apex develops into a tightly packed, helical, cluster of floral primordia resembling a cauliflower (Bowman, 1992; Kempin et al., 1995). The effect of *sin1* appears to be additive on *ap1*. Therefore, *sin1* is unlikely to directly regulate *AP1* activity. The effect of the *sin1-1* allele on the *ap1-1* mutant phenotype of the first flower is enhanced at 17°C, a temperature at which *ap1-1*, the most severe allele of *ap1* known, is more expressive (Bowman et al., 1993). These results indicate that both SIN1 and AP1 products are simultaneously required for early floral determination. Taken together, it appears that *SIN1* acts through a genetic mechanism different from that of *AP1* and *CAL*. One possibility is that SIN1 may directly interact with LFY because LFY and AP1 have been hypothesized to regulate parallel pathways (Shannon and Meeks-Wagner, 1993). As expected, *ap1 lfy* double mutations result in an enhanced effect on flowering time, especially in SD (Schultz and Haughn, 1993). Recent evidence, however, indicates that at least one component of LFY activity needs *AP1* gene for specifying early floral meristem identity (Weigel and Nilsson, 1995). *SIN1* may be necessary for the *AP1*-independent component of LFY. Alternatively, SIN1 may indirectly regulate flowering by controlling the general competence of the meristematic cells to respond to the activity of meristem identity genes (Weigel and Nilsson, 1975). Synergistic interaction has been reported between the weak *lfy-5* allele and *constans*, which results in late flowering (Putterill et al., 1995), suggesting that flowering time genes may directly or indirectly regulate activities of meristem identity genes such as *LFY*.

Tests of genetic models by epistasis analysis require the mutant alleles to be null. The allele *sin1-2* is weaker than *sin1-1* in its effect on ovule morphology because *sin1-1/sin1-2* heterozygotes show intermediate levels of ovule defect compared to either homozygous mutant (Golden, Ray and Ray, unpublished). Expressivity of the delayed flowering phenotype of *sin1-2*, however, is approximately equal to that of *sin1-1* and that of the *sin1-1/sin1-2* heterozygote. If ovule and meristem defects are pleiotropic effects of the same molecular lesion, then the meristem defect is more sensitive to an alteration of the SIN gene product than is the ovule defect, and the effect of the *sin1-1* allele on meristem transition is complete. Therefore, we suggest that the *sin1-1* allele can be adequately used for epistasis analysis. It is not known whether *tfl1-1* or *tfl1-2* are null alleles, and *ap1-1*, the strongest known allele, is not null. Therefore, all our conclusions based on epistasis analysis, as are most conclusions based on epistasis in *Arabidopsis*, are subject to future revision. The higher sensitivity of the meristem defect to a *sin1* mutational allele suggests that a sharper threshold of SIN1 activity is required for normal meristem transition than for normal ovule development. Alternatively, *SIN1* may encode a bifunctional product having organ-specific functional domains.

We thank Dr Charles S. Gasser for generously providing the *sin1-2* mutant strain. Seed stocks were also obtained from Nottingham (NW strains) and Ohio (CS strains) Arabidopsis Stock Centers. Comments from Dr Z. R. Sung and three anonymous reviewers significantly improved the manuscript. This work was partially supported by a Cooperative State Research Service Award from the US Department of Agriculture (92-37304-7966) to A. R., and by the Department of Biology, University of Rochester. During this work, J. D. L. worked without a salary.

REFERENCES

Alvarez, J., Guli, C. L., Lu, X.-H. and Smyth, D. R. (1992). *Terminal Flower*: A gene affecting inflorescence development in *Arabidopsis thaliana*. *The Plant J.* **2**, 103-116.

Bowman, J. L. (1992). Making cauliflower out of *Arabidopsis*: The specification of floral meristem identity. *Flowering Newslett.* **14**, 7-19.

Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E. M. and Smyth, D. R. (1993). Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* **119**, 721-743.

Colombo, L., Franken, J., Koetje, E., van Went, J., Dons, H. J. M., Angenent, G. C. and van Tunen, A. J. (1995). The petunia MADS box gene *FBP11* determines ovule identity. *The Plant Cell* **7**, 1859-1868.

Coupland, G. (1995). Genetic and environmental control of flowering time in *Arabidopsis*. *Trends Genet.* **11**, 393-397.

Clarke, J. H. and Dean, C. (1994). Mapping *FRI*, a locus controlling flowering time and vernalization response in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **242**, 81-89.

Gaiser, J. C., Robinson-Beers, K. and Gasser, C. S. (1995). The Arabidopsis *SUPERMAN* gene mediates asymmetric growth of the outer integument of ovules. *The Plant Cell* **7**, 333-345.

Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1994). Regulation of the Arabidopsis floral homeotic gene *APETALA1*. *Cell* **76**, 131-143.

Herr, J. M. (1995). The origin of the ovule. *Am. J. Bot.* **82**, 547-564.

Huala, E. and Sussex, I. M. (1992). *Leafy* interacts with floral homeotic genes to regulate *Arabidopsis* floral development. *The Plant Cell* **4**, 901-913.

Irish, V. and Sussex, I. M. (1990). Function of the *apetala-1* gene during *Arabidopsis* floral development. *The Plant Cell* **2**, 741-753.

Kempin, S. A., Savidge, B. and Yanofsky, M. F. (1995). Molecular basis of the *cauliflower* phenotype in *Arabidopsis*. *Science* **267**, 522-525.

Koornneef, M. K., Hanhart, C. J. and Van der Veen, J. H. (1991). A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **229**, 57-66.

Lang, J. D., Ray, S. and Ray, A. (1994). *sin1*, a mutation affecting female fertility in *Arabidopsis*, interacts with *mod1*, its recessive modifier. *Genetics* **137**, 1101-1110.

Lee, I., Aukerman, M. J., Gore, S. L., Lohman, K. N., Michaels, S. D., Weaver, L. M., John, M. C., Feldmann, K. A. and Amasino, R. M. (1994). Isolation of *LUMINIDIPENDENS:* A gene involved in the control of flowering time in *Arabidopsis*. *The Plant Cell* **6**, 75-83.

Ma, H. (1994). The unfolding drama of flower development: recent results from genetic and molecular analyses. *Genes Dev.* **8**, 745-756.

Mandel, M. A., Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. *Nature* **360**, 273-277.

Mandel, M. A. and Yanofsky, M. F. (1995). A gene triggering flower formation in *Arabidopsis*. *Nature* **277**, 522-524.

Martinez-Zapater, J. M., Coupland, G., Dean, C., Koornneef, M. (1994). The transition to flowering in *Arabidopsis*. In *Arabidopsis*, (ed. Meyerowitz, E. M. and Somerville, C.), pp.403-433, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Meyerowitz, E. M. (1994). Flower development and evolution: New answers and new questions. *Proc. Natl. Acad. Sci. USA* **91**, 5735-5737.

Modrusan, Z., Reiser, L., Feldmann, K. A. Fischer, R. L. and Haughn, G. W. (1994). Homeotic transformation of ovules into carpel-like structures in *Arabidopsis*. *The Plant Cell* **6**, 333-349.

Poethig, S. C. (1990). Phase change and the regulation of shoot morphogenesis in plants. *Science* **150**, 923-930.

Putterill, J., Robson, F., Lee, K., Simon, R. and Coupland, G. (1995). The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* **80**, 847-857.

Ray, A., Robinson-Beers, K., Ray, S., Baker, S. C., Lang, J. D., Preuss, D., Milligan, S. B. and Gasser, C. S. (1994). The *Arabidopsis* floral homeotic gene BELL (*BEL1*) controls ovule development through negative regulation of AGAMOUS (*AG*) gene. *Proc. Natl. Acad. Sci. USA* **91**, 5761-5765.

Reiser, L., and Fischer, R. L. (1993). The ovule and the embryo sac. *The Plant Cell* **5**, 1291-1301.

Reiser, L., Modrusan, Z., Margossian, L., Samach, A., Ohad, N., Haughn, G. W. and Fischer, R. L. (1995). The *BELL1* gene encodes a homeodomain protein involved in pattern formation in the Arabidopsis ovule primordium. *Cell* **83**, 735-742.

Robinson-Beers, K., Pruitt, R. E. and Gasser, C. S. (1992). Ovule development in wild-type *Arabidopsis* and two female sterile mutants. *Plant Cell* **4**, 1237-1250.

Sakai, H., Medrano, L. J. and Meyerowitz, E. M. (1995). Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. *Nature* **378**, 199-203.

Schultz, E. A. and Haughn, G. W. (1993). Genetic analysis of the floral initiation process (FLIP) in *Arabidopsis*. *Development* **119**, 745-765.

Shannon, S. and Meeks-Wagner, D. R. (1991). A mutation in the Arabidopsis *TFL1* gene affects inflorescence meristem development. *The Plant Cell* **3**, 877-892.

Shannon, S. and Meeks-Wagner, D. R. (1993). Genetic interactions that regulate inflorescence development in Arabidopsis. *The Plant Cell* **5**, 639-655.

Stebbins, G. L. (1974). *Flowering Plants: Evolution Above the Species Level*, pp. 199-245.Cambridge, MA: Harvard University Press.

Sung, Z. R., Belachew, A., Shunong, B. and Bertrand-Garcia, R. (1992). *EMF*, an *Arabidopsis* gene required for vegetative shoot development. *Science* **25**, 1645-1649.

Taylor, T. N. and Taylor, E. L. (1993). *The Biology and Evolution of Fossil Plants*. Englewood Cliffs, NJ: Prentice Hall.

Weigel, D. (1995). Genetics of flower development: From floral induction to ovule morphogenesis. *Ann. Rev. Genet.* **29**, 19-39.

Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F. and Meyerowitz, E. M. (1992). *LEAFY* controls floral meristem identity in *Arabidopsis*. *Cell* **69**, 843-859.

Weigel, D. and Meyerowitz, E. M. (1993). Activation of floral homeotic genes in *Arabidopsis*. *Science* **261**, 1723-1726.

Weigel, D. and Meyerowitz, E. M. (1994). The ABCs of floral homeotic genes. *Cell* **78**, 203-209.

Weigel, D. and Nilsson, O. (1995). A developmental switch sufficient for flower initiation in diverse plants. *Nature* **277**, 495-500.

Wilson, R. N., Heckman, J. W., and Somerville, C. R. (1992). Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. *Pl. Physiol.* **100**, 403-408.

Yang, C.-H., Chen, L.-J. and Sung, Z. R. (1995). Genetic regulation of shoot development in *Arabidopsis*: Role of the *EMF* gene. *Dev. Biol.* **169**, 421-435.

Zagotta, M. T., Shannon, S., Jacobs, C. and Meeks-Wagner, D. R. (1992). Early flowering mutants of *Arabidopsis thaliana*. *Austral. J. Genet.* **19**, 411-418.

(Accepted 24 May 1996)